
**Titre du document / Document title**
An alternative mechanism of bioluminescence color determination in firefly luciferase

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**Résumé / Abstract**
Beetle luciferases (including those of the firefly) use the same luciferin substrate to naturally display light ranging in color from green (λ[m][a][x] ~530 nm) to red (λ[m][a][x] ~635 nm). In a recent communication, we reported (Branchini, B. R., Murtiashaw, M. H., Magyar, R. A., Portier, N. C., Ruggiero, M. C., and Stroh, J. G. (2002) J. Am. Chem. Soc. 124, 2112-2113) that the synthetic adenylate of firefly luciferin analogue D-5,5-dimethylluciferin was transformed into the emitter 5,5-dimethyloxyluciferin in bioluminescence reactions catalyzed by luciferases from Photinus pyralis and the click beetle Pyrophorus plagiophthalamus. 5,5-Dimethyloxyluciferin is constrained to exist in the keto form and fluoresces mainly in the red. However, bioluminescence spectra revealed that green light emission was produced by the firefly enzyme, and red light was observed with the click beetle protein. These results, augmented with steady-state kinetic studies, were taken as experimental support for mechanisms of firefly bioluminescence color that require only a single keto form of oxyluciferin. We report here the results of mutagenesis studies designed to determine the basis of the observed differences in bioluminescence color with the analogue adenylate. Mutants of P. pyralis luciferase putative active site residues Gly246 and Phe250, as well as corresponding click beetle residues Ala243 and Ser247 were constructed and characterized using bioluminescence emission spectroscopy and steady state kinetics with adenylate substrates. Based on an analysis of these and recently reported (Branchini, B. R., Southworth, T. L., Murtiashaw, M. H., Boije, H., and Fleet, S. E. (2003) Biochemistry 42, 10429-10436) data, we have developed an alternative mechanism of bioluminescence color. The basis of the mechanism is that luciferase modulates emission color by controlling the resonance-based charge delocalization of the anionic keto form of the oxyluciferin excited state.

**Revue / Journal Title**
Biochemistry (Biochemistry) **ISSN** 0006-2960

**Source / Source**
2004, vol. 43, n°23, pp. 7255-7262 [8 page(s) (article)]

**Langue / Language**
Anglais

**Editeur / Publisher**
American Chemical Society, Washington, DC, ETATS-UNIS (1962) (Revue)

**Localisation / Location**
INIST-CNRS, Cote INIST : 9758, 35400011363786.0040



N° notice refdoc (ud4) : 15864633